LBNL-47628 Abs.

This abstract was prepared for the 4th Annual NABIR PI Workshop to be held at the Arlie Conference Center, Warrenton, Virginia, March 12-14, 2001.

Prepared: December 8, 2000

Metabolic engineering of microorganisms for actinide and heavy metal precipitation

Jay D. Keasling[1], Douglas S. Clark[1], and Heino Nitsche[2,3]
[1]Departments of Chemical Engineering and [2]Chemistry, University of California
[3]The Glenn T. Seaborg Center, Lawrence Berkeley National Laboratory
Berkeley, CA 94720

Heavy metals and actinides are significant problems at a number of DOE sites and industrial locations in the U.S. Many of these sites contain heavy metals, actinides and organics. Due to the costs associated with excavating, transporting, and remediating contaminated sediments at remote locations, an economically viable solution is to mineralize the organic contaminants *in situ* and immobilize the metals and actinides to prevent movement to other locations. There are few reports in the literature of organisms capable of all of these functions. Besides their potential use *in situ*, these organisms should find use in treating wastes tanks at such sites as Hanford that contain mixed organics, metals, and actinides.

During the previous grant period, we isolated and characterized a novel strain of *Pseudomonas aeruginosa* from a deep-sea hydrothermal vent capable of removing high levels of cadmium from solution by reducing thiosulfate to sulfide and precipitating cadmium as cadmium sulfide on the cell wall. To improve upon this system, we successfully engineered *Escherichia coli*, *Pseudomonas aeruginosa*, and *Pseudomonas putida* to remove heavy metals and actinides from solution and immobilize them on the cell wall. For precipitation of cadmium, zinc, lead, and other metals that form strong sulfide complexes, we developed two systems for aerobic sulfide production: (1) expression of serine acetyl transferase and cysteine desulfhydrase in *E. coli* for overproduction of cysteine and subsequent conversion to sulfide; and (2) expression of thiosulfate reductase in *E. coli* and *P. putida* for reduction of thiosulfate to sulfide. The *P. putida* system was shown to allow simultaneous heavy metal precipitation and organics degradation. For precipitation of actinides as complexes of phosphate, we overexpressed polyphosphate kinase in *E. coli* and *P. aeruginosa* to enable these organisms to accumulate high levels of polyphosphate during phosphate excess and exopolyphosphatase for polyphosphate degradation and concomitant secretion of phosphate from the cell. All of these systems were shown to be capable of removing relatively high levels of metals from solution and have potential for metal and actinide removal from contaminated waste streams or immobilizing these elements *in situ.*

The goal of our work is to engineer heavy metal and actinide precipitation in two microorganisms that will be relevant for treatment of DOE sites contaminated with heavy metals, actinides, and/or organics: *Pseudomonas aeruginosa* and *Deinococcus radiodurans*. Specifically, we propose (1) to engineer polyphosphate synthesis and degradation into *Deinococcus radiodurans* and *P. putida* for removal of uranium(VI) and plutonium(VI, V); (2) to engineer aerobic sulfide production into *D. radiodurans* for removal of cadmium, zinc, and lead; and (3) to test removal of actinides; actinides and heavy metals; and actinides, heavy metals, and organics using the engineered organisms.

Acknowledgements: This work is supported by the U.S. Department of Energy under Contract No. DE-AC03-76SF00098